Journal of Cell Science 104, 583-593 (1993)
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Abnormal anaphase resolution (*aar*): a locus required for progression through mitosis in *Drosophila*

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SUMMARY

We describe a new mitotic locus of *Drosophila melanogaster* required for the progression through mitosis in the syncytial embryo and in late larval development. The locus *aar* (*a*bnormal *a*naphase *r*esolution) maps to the cytological interval 85E7-F16 and was identified by two alleles. The *aar*^{1} allele causes pupal lethality. Larval neuroblasts show an elevated mitotic index with high chromosome condensation and stretched and lagging chromatids during anaphase. *aar*^{2} produces fully viable but sterile females. *aar*^{1}/*aar*^{2} females lay eggs that develop mitotic figures with similar abnormalities to those observed in neuroblasts. Indirect immunofluorescence of these embryos indicates that the centrosome cycle appears normal, although some abnormal spindle microtubules can be seen during mitosis.

Key words: mitosis, mutant, metaphase, anaphase, *aar*, *Drosophila*

INTRODUCTION

The value of applying genetics to the study of complex cellular processes is derived from its ability to identify important, but relatively low-abundance components, and to provide a hint of their function. Genetic approaches have identified a number of loci in *Drosophila* whose products are essential for mitosis (reviewed by Gatti and Baker, 1989; Glover, 1989).

In general, a given mitotic mutant in *Drosophila* manifests one of two lethal phenotypes: embryonic lethality in eggs laid by a homozygous mutant mother; and late larval or pupal lethality of a homozygous mutant individual. The maternal-effect lethality of a mitotic mutant is due to the near total dependence of the extremely rapid nuclear divisions of the early embryos on maternally supplied mitotic machinery. Post-embryonic mitotic failure leads to lethality at pupation because mitoses are largely confined to the imaginal tissues of the larvae, which are only needed at pupation. The specific lethal phase of a mitotic mutant depends therefore, on both the particular role the gene product plays in mitotis and the severity (leakiness) of the allele.

A number of *Drosophila* genes have been identified whose mutations affect mitotis. Some of these genes appear to be required only during the syncytial embryo in which there are 13 rounds of mitosis before formation of the cellular blastoderm (Zalokar and Erk, 1976; Foe and Alberts, 1983). These include *gnu* (Freeman et al., 1986), *abc* (Underwood et al., 1990; Vessey et al., 1991), *plu* and *png* (Shamanski and Orr-Weaver, 1991) and *fs(1)Ya* (Lin and Wolfner, 1991). The product of *gnu* is thought to be required only during early embryogenesis and mutations in this locus cause inappropriate DNA synthesis in unfertilized eggs (Freeman and Glover, 1987). Also, the products of *plu* and *png* seem to be required to maintain repression of DNA replication, regulating entry into S-phase at fertilization (Shamanski and Orr-Weaver, 1991). *fs(1)Ya* is also required early in development and it has been shown to encode a nuclear envelope component (Lin and Wolfner, 1991).

Other mitotic genes have been identified which encode proteins thought to be required soon after blastoderm formation. At nuclear division cycle 14, synchrony is lost, and cells divide within a series of mitotic domains (Foe, 1989). Two genes have been identified which need to be expressed *de novo* at this stage to allow cell cycle progression within

these mitotic domains. The zygotic expression of cyclin A (Lehner and O'Farrell, 1989) and *string* (Edgar and O'Farrell, 1989; Jimenez et al., 1990), is required to regulate the length of G_2 phase within these newly cellularized embryonic cells (Edgar and O'Farrell, 1990), whereas zygotic expression of cyclin A is needed for subsequent cell division (Lehner and O'Farrell, 1989).

Several other genes have been identified because mutations affect the behaviour or morphology of chromosomes in third larval instar neuroblasts. These include, mutations such as: *asp*, which affect spindle function (Ripoll et al., 1985); *mgr*, which might be required for spindle pole function (Gonzalez et al., 1988); *sqh*, which encodes the regulatory light chain of nonmuscle myosin and is involved in cytokinesis (Karess et al., 1991); *ck19*, which encodes a type I protein phosphatase (Axton et al., 1990); and *rough deal* (Karess and Glover, 1989), which is thought to be involved in the mechanism that assures the proper release of sister chromatids.

Some of these genes have demonstrable roles in both embryonic and larval mitosis. *polo* (Sunkel and Glover, 1988) encodes a protein kinase homologue (Llamazares et al., 1991). Leaky alleles like $polo^1$ are viable but cause female sterility, giving rise to abnormal nuclear multiplication stages. They also show a range of mitotic abnormalities associated with a delay in the metaphase-to-anaphase progression, as well as non-disjunction in male meiosis (Sunkel and Glover, 1988). The larval lethality in $polo^2$ reflects the effects of stronger mutant allele upon cell division at this developmental stage. *lodestar* (Girdham and Glover, 1991) encodes a putative nucleoside triphosphate binding protein which causes chromosome tangling and breakage at anaphase.

In this paper, we describe the identification of *a*bnormal *a*naphase *r*esolution (*aar*), a gene whose product is required for normal mitosis in both embryos and larvae. Larvae homozygous for aar^1 show an accumulation of mitotic cells with abnormal, highly condensed metaphase and anaphase figures, irregular chromatid condensation and lagging or stretched chromatids during anaphase. A second allele, aar^2, is homozygous viable but mutant females lay eggs whose early mitotic divisions are severely disrupted. We present evidence that the abnormal mitoses of *aar* mutants are associated with altered spindle morphology and a general delay in progression through metaphase and anaphase.

MATERIALS AND METHODS

Genetic variants

The allele aar^1 was isolated from a collection of 3rd chromosome recessive late larval lethal mutations induced by P-M mutagenesis. Females of M-cytotype isogenic for a 3rd chromosome marked with *red e* were crossed with Harwich P-males and stocks established by usual means (Kidwell, 1986). The aar^1 stock was identified for further study because of abnormal mitotic figures observed in neuroblasts of homozygous third-instar larvae. All stocks used for phenotypic analysis of third-instar larval brains were balanced over TM6, *Tb e ca*. The aar^2 allele was identified in a complementation test with aar^1 as a recombinant from $Df(3R)G42^{P}R36^{D}$, a synthetic deficiency segregated by translocations which delete cytogenetic region 85E-86B (Lindsley et al., 1972; Jürgens et al., 1984). We made trans-heterozygotes between this recombinant stock and aar^1, and found that these individuals were viable and third-instar larval neuroblasts showed no abnormal phenotype. However, trans-heterozygous females were sterile and most of the embryos laid never reached cellularization. Analysis of polytene chromosomes from heterozygotes with either aar^1 or Oregon-R showed that the recombinant stock had no cytological abnormalities (data not shown). This recombinant chromosome was further cleaned through recombination with a multiply marked stock and tested for complementation with Df(3R)by10 and Df(3R)by62 (see Fig. 3B, below). As a result we isolated a recombinant (*ru st cu sr ca*) which contains the region between *st* and *cu* from the original chromosome and mapped the sterile phenotype in the same cytogenetic region as aar^1. All other stocks were described by Lindsley and Grell (1968) or Lindsley and Zimm (1992). All stocks were grown at 25˚C under standard culture conditions and media.

Neuroblast preparations

Cytological preparations were made from late third-instar larvae as described by Sunkel and Glover (1988) except for Hoechst 33258-labelled preparations. For these preparations, brains were dissected in saline and then transferred directly to 45% acetic acid for 15 s after which they were placed in a drop of 60% acetic acid on a siliconized coverslip. The fixed brain was spread by picking the coverslip with a slide, excess liquid was blotted and the preparation squashed. The slide was quickly frozen in liquid nitrogen, the coverslip removed and the preparation allowed to air dry. Cells were stained by placing a drop of Hoechst (1 μg/ml in saline) for 1-3 min and then washed extensively in saline before drying. The preparation was mounted in 2.5% isopropylgallate in 85% glycerol and observed in a Zeiss epifluorescence microscope. Quantification of mitotic figures was performed as by Gonzalez et al. (1991) except that we used a smaller microscope field (100 × 10). Hypotonic shock was performed by placing whole brains in 0.5% sodium citrate for 5 min prior to fixation.

In situ hybridization

In situ hybridization on polytene chromosomes was performed using tritiated probes as described by Pardue (1986).

Fixation and staining of embryos

Embryos were fixed and stained as described by Freeman et al., (1986). Taxol was used to stabilize microtubules as described by Karr and Alberts (1986).

Antibodies

The antibodies used in this study were the following: anti-centrosome antibody, RB188 (Whitfield et al., 1988) and anti-β-tubulin antibody (Amersham). Rhodamine- and fluorescein-conjugated second antibodies were bought, respectively, from Jackson Immunoresearch and Amersham.

Polytene chromosomes

Salivary glands from crawling third-instar larvae were dissected in saline, fixed for 30 s in 45% glacial acetic acid, stained for 5 min in 2% lactic-acetic orcein and squashed in lactic acid:water:acetic acid (1:2:3, by vol.) under a siliconized coverslip. The breakpoints of all deficiencies used in this study were confirmed using Df/+ heterozygote chromosomes.

RESULTS

Mitotic phenotypes in larval neuroblasts

The somatic mitotic abnormalities caused by the mutant

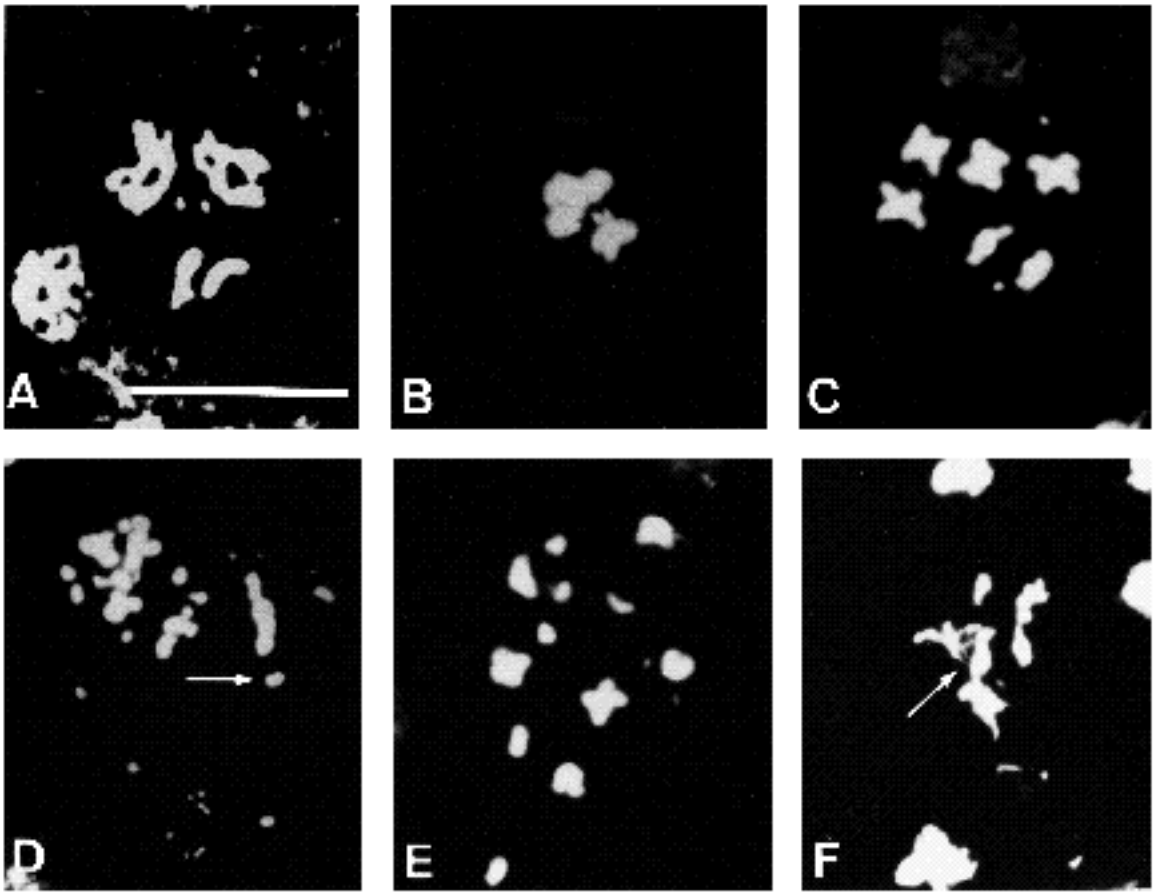

Fig. 1. Metaphase figures in wild-type (A) and *aar*[1] homozygotes of larval neuroblasts (B-F) stained with Hoechst 33258. Wild-type female metaphase plate (A). Metaphase figure showing excessive chromosome condensation (B) and similar metaphase figure after hypotonic shock treatment (C). Note that highly condensed chromosomes do not appear to be associated with chromosome fragmentation. Polyploid metaphase showing extreame chromosome condensation (arrow in D) and a similar polyploid figure after hypotonic shock (E). Abnormal metaphase figure showing irregular chromatin condensation (arrow in F). Bar, 6 μm.

allele *aar*[1] were studied in the diploid neuroblasts of the homozygous third-instar larvae. The mitotic alterations found were confined to the degree of chromosome condensation and their ploidy (Fig. 1) and to the structure and organization of the anaphase (Fig. 2) figures.

Three types of abnormal metaphase figures were observed (Fig. 1A). (1) The vast majority of metaphase figures show excessive chromosome condensation (Fig. 1B). (2) We also observed highly polyploid and disorganized metaphase figures, which are also characterized by excessive chromosome condensation (Fig. 1D). Neither diploid nor polyploid metaphases appear to be associated with chromosome fragmentation, and when preparations were subjected to hypotonic shock, chromosomes seemed to be intact (Fig. 1C,E). (3) We also found disorganized metaphase figures in which portions of chromatids show irregular condensation (Fig. 1F).

However, the most striking phenotype was found amongst anaphase figures (Fig. 2). A large proportion of anaphases are abnormal as compared to the wild-type control (Fig. 2A). Three types of abnormalities can be found. First, anaphases in which isolated chromatids appear to be lagging and left in the mid-zone oriented parallel to the spindle (Fig. 2B,C). Secondly, anaphases that show stretched chromatids between the two poles (Fig. 2D-F). In some of these anaphases, stretched chromatin seems to emanate from a chromatin mass left in the mid-zone (Fig. 2E). And, finally, most of these anaphase figures have chromatids that appear with a variable but high degree of condensation (Fig. 2C-F).

Quantification of mitotic figures

A quantitative analysis of the different abnormal phenotypes observed in *aar*[1] cells is presented in Table 1. From these results we can conclude that in homozygous mutant cells there is a high proportion of metaphase figures which show high levels of chromosome condensation (73.9%). There is some elevation in the number of polyploid cells (3.8%). The results also show that only 16.6% of anaphases appear normal in mutant brains, and that most abnormal anaphases show either lagging (53.4%) or stretched chromatids (36.1%), and a significant proportion (50.0%) display high chromatin condensation. In the *aar*[1]/Df heterozygotes, the mitotic abnormalities are equivalent to those described above. However, some changes in the proportion of individual phenotypes are apparent. These include a higher pro-

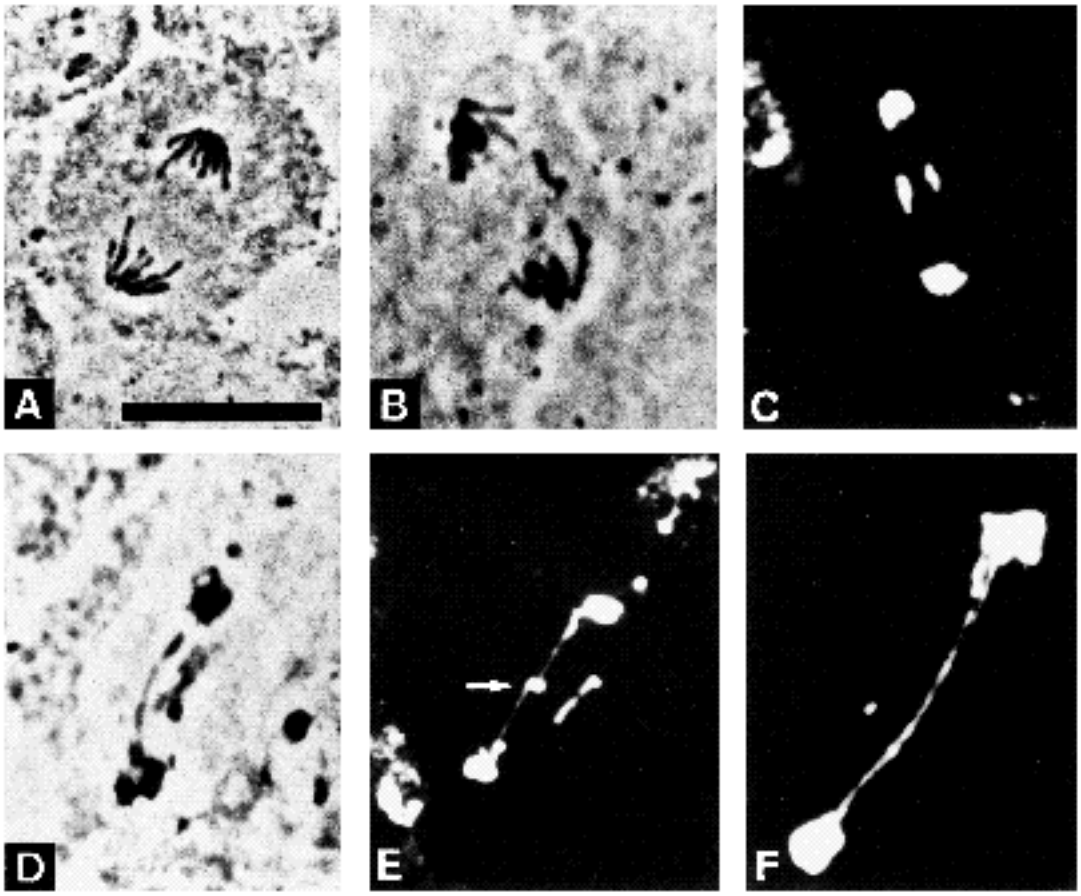

Fig. 2. Anaphase figures in wild-type (A) and *aar*^{1} homozygote larval neuroblasts (B-F). Preparations A,B,D were stained with orcein and preparations C,E,F with Hoechst 33258. Anaphase figures showing lagging chromatids (B-F) and stretched chromatids (D-F). Anaphase figure showing a lagging chromatin mass in the mid-zone, part of which seem to be stretched towards the two poles (arrow in E). Most anaphases are also associated with high levels of chromosome condensation (C-F). Bar, 6 μm.

Table 1. Percentage of metaphase or anaphase figures in *aar*^{1}/*aar*^{1}, *aar*^{1}/Df and wild-type control third-instar larvae

		Genotypes		
Mitotic stage	Phenotype	OR	*aar*^{1}/*aar*^{1}	*aar*^{1}/Df
Metaphase	Normal	74.0	8.4	8.0
	High condensation	25.0	73.9	63.9
	Irregular condensation	<0.1	8.4	7.1
	Polyploid	<0.1	3.8	11.3
	Disorganized	1.0	4.1	8.0
	Aneuploid	<0.1	1.1	2.1
Anaphase	Normal	83.0	16.6	11.3
	Stretched	<0.1	36.1	23.3
	Lagging	4.0	53.4	27.9
	High condensation	9.0	50.0	69.8

Numbers are expressed as percentage over total and due to the fact that a mitotic figure might show mixed phenotypes the numbers will not add up to 100.

portion of polyploid metaphases (11.3%), reduced proportion of normal anaphases (11.3%) and stretched (23.3%) or lagging chromatids (27.9%), while the proportion of highly condensed anaphases is increased (69.8%).

We have quantified the mitotic activity of the *aar*^{1} third-instar larval neuroblasts and compared it with that of a wild-type Oregon-R strain. The results are summarized in Table 2. The mitotic index is much higher in *aar*^{1} homozygous cells (2.35), which showed elevated levels of both metaphase (1.6) and anaphase (0.72) figures, relative to wild-type controls of, respectively, 1.32, 1.0 and 0.32. However, the frequency of metaphase figures of *aar*^{1} cells (0.69) is lower than that of wild-type cells (0.76), while the frequency of anaphases of *aar*^{1} cells (0.31) is higher than that of wild-type cells (0.24). The mitotic activity in *aar*^{1}/Df heterozygote neuroblast cells was also quantified. The results (Table 2) indicate that *aar*^{1} is probably a leaky mutation, since a more severe mitotic phenotype is observed in this cells when compared to *aar*^{1} homozygotes. The total mitotic index (1.76) is higher than normal, probably due to the high number of metaphases per field (1.49), although there is a reduction in the number of anaphases per field (0.27). This is also apparent from the higher frequency of metaphases (0.85) and a lower frequency of anaphases (0.15). The results indicate that in *aar*^{1} homozygotes and *aar*^{1}/Df heterozygotes there is a delay in the progression through mitosis.

Two phenotypes can therefore be assigned to the *aar*^{1}

Table 2. Quantification of mitotic activity in *aar*^{1}/*aar*^{1}, *aar*^{1}/Df and wild-type third-instar larvae

Genotype	Mitotic* figures	Fields†	Number of metaphases	Number of anaphases	Mitotic index	Metaphase per field	Anaphase per field	Frequency metaphases	Frequency anaphases
OR	1062	804	804	258	1.32	1.0	0.32	0.76	0.24
aar^1/*aar*^{1}	1728	734	1186	532	2.35	1.6	0.72	0.69	0.31
aar^1/Df‡	1405	800	1190	215	1.76	1.49	0.27	0.85	0.15

*15 brains were scored for each genotype.
†Microscopic area observed under phase-contrast (100×10).
‡Df(3R)by62.

mutation: (1) abnormal behaviour of chromosomes (lagging, stretching and overcondensing), and (2) delay in metaphase-to-anaphase transit time.

Localization and genetic mapping of the *aar* locus

The original mutant stock carrying the *aar*^{1} allele was repeatedly crossed with multiple balancer stocks so as to replace all chromosomes except the 3rd. In order to map the *aar*^{1} allele, an isogenic line *red e aar*^{1} was crossed with the multiply marked chromosome *ru h th st cu sr ca*. Several recombinant chromosomes were selected and used to localize *aar*^{1} to the interval between *st* and *red*. A *aar*^{1} recombinant chromosome carrying *ru h th st sr ca* was used for *in situ* hybridization with a P-element probe (O'Hare and Rubin, 1983) and it was shown to contain five insertions localized between 85F and 87C (Fig. 3A). Further meiotic recombination of this chromosome was not attempted, since each of the five insertions were very close together. To determine whether any of the insertions was indeed associated with the *aar*^{1} phenotype we tested this chromosome for complementation with a number of known deletions which would uncover, one at a time, all five insertions (Fig. 3B). The results showed that only the Df(3R)by62 was unable to complement both the lethality and the mitotic phenotype in the original, isogenic or the recombinant *ru h th st sr ca* mutant lines (Fig. 3B). Since the Df(3R)by10 was able to fully complement all the mutant chromosomes we can assign *aar*^{1} to interval 85E7-F16. We can also suggest that the *aar*^{1} mutation might be associated with the P-element located in 85F (Fig. 3A).

Identification of the female sterile allele *aar*^{2}

In the course of the genetic studies aimed at the localiza-

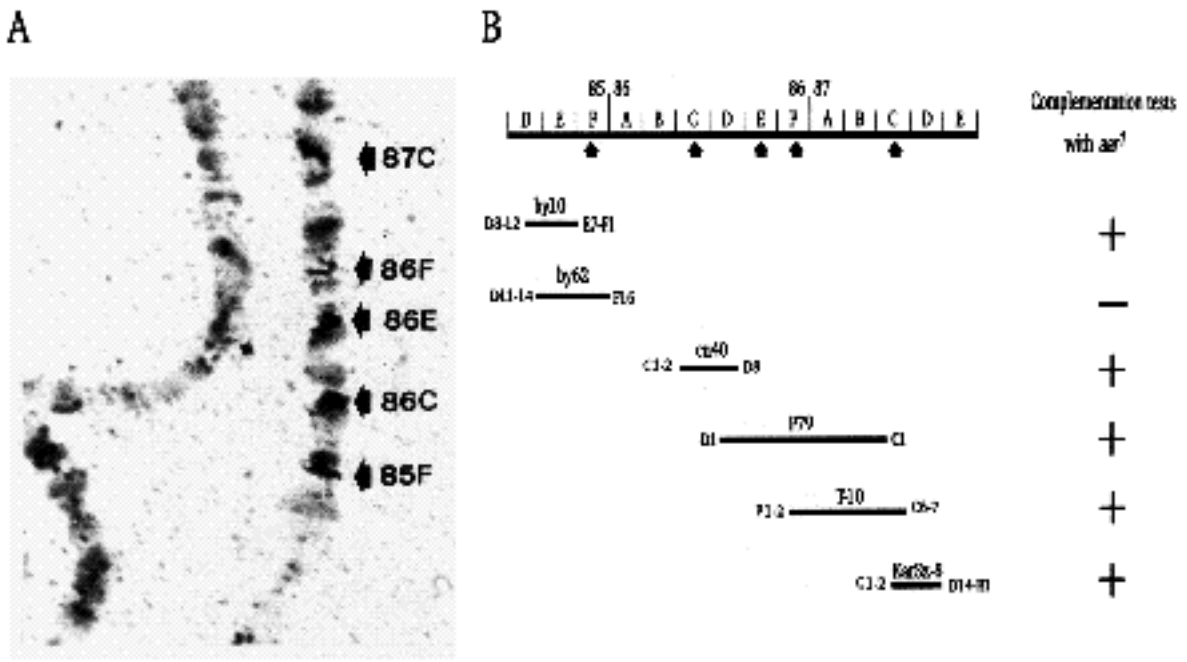

Fig. 3. Cytological mapping of the P-element associated with *aar*^{1}. (A) *In situ* hybridization of a homozygote *aar*^{1} polytene chromosome with a P-element probe revealing the presence of five P-element insertions. (B) The top diagram shows part of the 3rd chromosome including the cytogenetic region 85D;87E where P-element insertions were found (large arrows). Below, the deficiencies used to uncover the cytogenetic region where *aar*^{1} and *aar*^{2} were mapped are represented by horizontal bars. Above each bar is indicated the designation of the deletion, and the capital letters and numbers represent the cytological breakpoints. The results of genetic complementation tests between *aar*^{1} and each one of the deficiency stocks is indicated on the right. (+) indicates full complementation and (−) indicates inability to complement the late larval lethality and the mitotic phenotype.

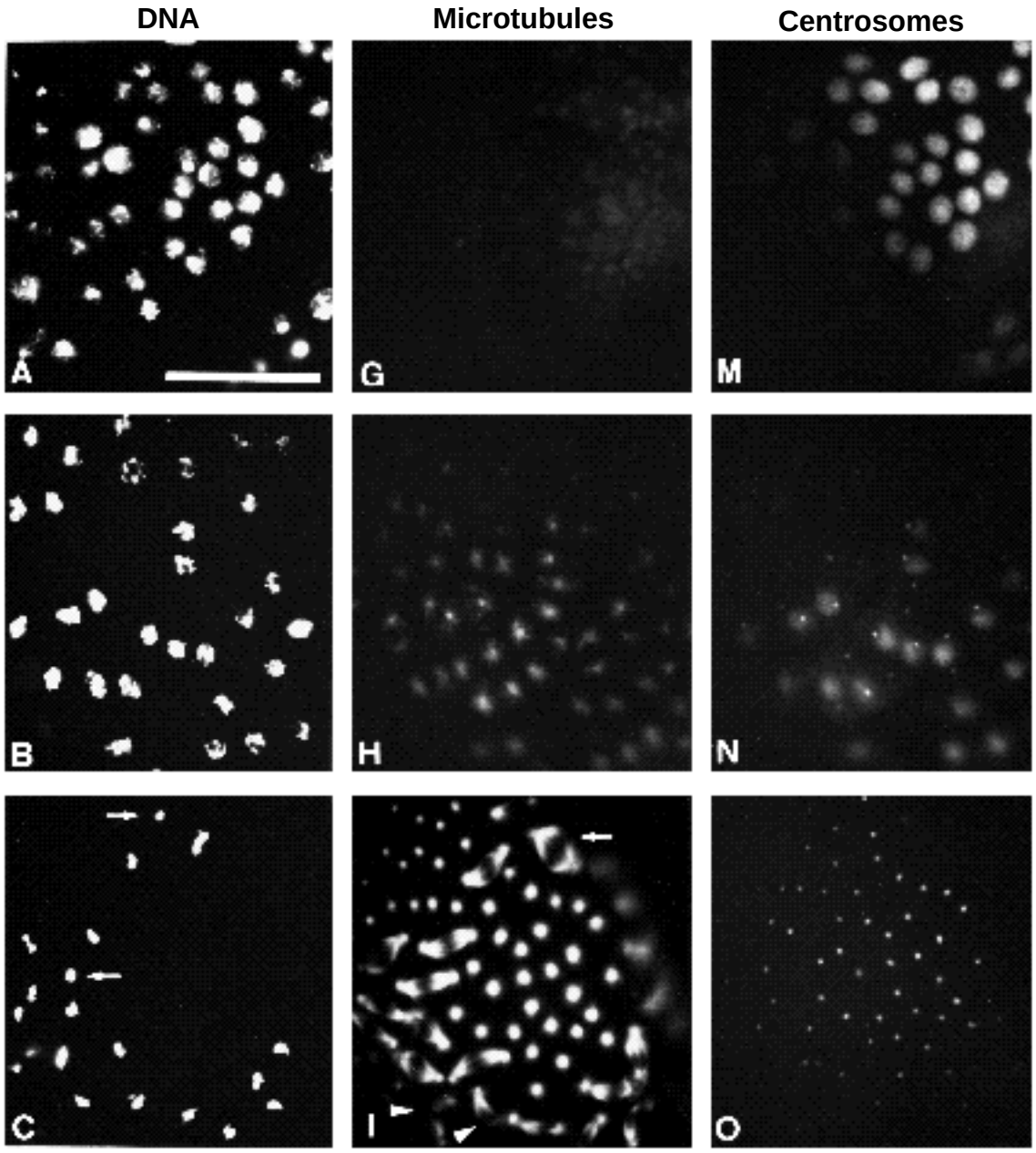

Fig. 4. Indirect immunofluorescence of embryos laid by *aar*^{1}/*aar*^{2} females. Embryos were labelled simultaneously with Hoechst 33258 to visualize DNA (A-F), anti-tubulin antibody to assess spindle microtubule organization (G-L) and anti-centrosome antibody (M-R). The mitotic figures shown in the six fields represent different stages of mitosis: interphase (A), prophase (B), metaphase (C), anaphase (D,E) and telophase (F). Highly condensed metaphase plates (arrows in C). Anaphase with a lagging chromatid (arrow in D). Telophase with lagging and stretched chromatid (arrow in F). Abnormally broad (arrow in I) and wavy spindles (arrowheads in I). Mitotic spindles with shared centrosomes (arrows in K and Q). Tripolar spindles composed of a main axis and microtubules nucleated by a centrosome from an adjacent cytoplasmic island (arrowheads in K and Q, and arrow in L). Bar, 40 μm.

tion of the P-element, which could be responsible for the *aar*^{1} mutant phenotype, we came across a second allele, *aar*^{2} (see Materials and Methods).

Since this allele does not show abnormal larval development but affects only early embryonic divisions we proceeded to test sterility in various allelic combinations. The results are shown in Table 3. These results indicate that the sterile mutation is an allele of *aar*^{1} and we have therefore designated it *aar*^{2}. The data indicate that in crosses between *aar*^{2} homozygotes or hemizygotes over Df(3R)by62, irrespective of which individual contributes the paternal genome, no adult escapers can be found. However, if males of either genotype were crossed to wild-type females, normal progeny were always obtained. This indicates that *aar*^{2} is purely maternal in its ability to cause abnormal embryonic development. The results also show that *aar*^{1} is

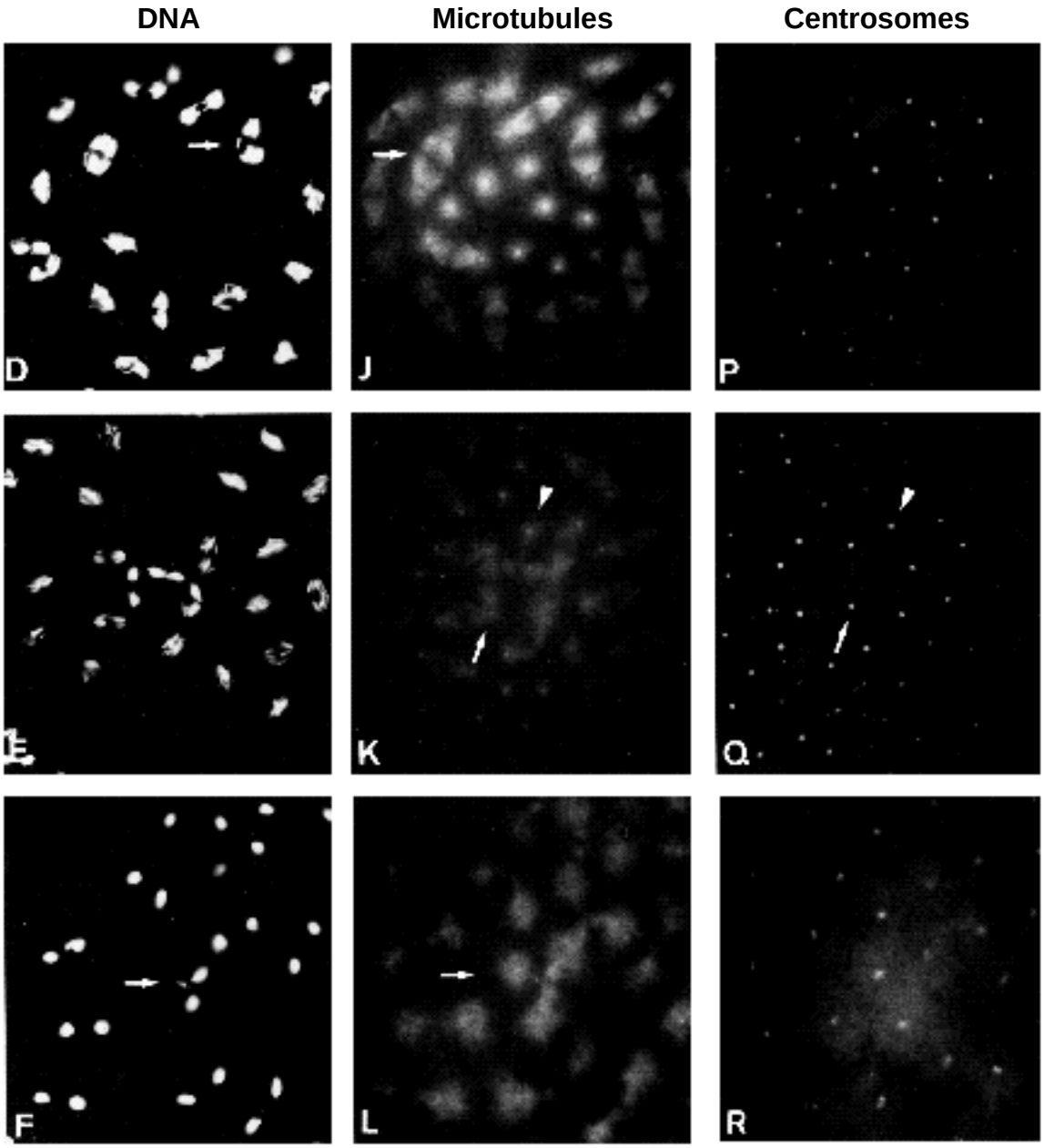

Fig. 4

not equivalent with respect to sterility as compared to *aar*^{2}. In all combinations in which the female is a trans-heterozygote *aar*^{1}/*aar*^{2}, a small proportion of adults was obtained (2-10% of eggs laid).

Table 3. Sterility test crosses between different combinations of *aar* alleles and a deficiency for the locus

	Male genotype			
Female genotype	*aar*^{1}/*aar*^{2}	*aar*^{2}/*aar*^{2}	*aar*^{2}/Df	+/+
aar^1/*aar*^{2}	5	3	2	10
aar^2/*aar*^{2}	0	0	0	0
aar^2/Df	0	0	0	0
+/+	>90	>90	>90	>90

Numbers indicate viable adults as a percentage of laid eggs.
Df, Df(3R)by62.

Early embryonic phenotype of *aar* mutants

We have studied the phenotype of early embryos produced from females of genotypes *aar*^{1}/*aar*^{2}, *aar*^{2}/*aar*^{2} and *aar*^{2}/Df(3R)by62, and characterized the cycles of nuclear multiplication together with the state of microtubule (tubulin) and centrosomal organization.

The results obtained for embryos derived from *aar*^{1}/*aar*^{2} females are shown in Fig. 4. The series of microphotographs represent different stages of the mitotic cycle before cellularization. It can be seen that in these embryos, there is an uneven distribution of nuclei throughout the cortex of the egg showing many spaces in which no nuclei can be found (Fig. 4A-F). However, these nuclei-free regions are always associated with microtubule asters (Fig. 4G-L), which are nucleated by centrosomes located in well-defined cytoplasmic islands (Fig. 4M-R). Nevertheless,

most nuclei do appear to retain their mitotic synchrony (Fig. 4A-F). In some cases, we do find telophase figures in which a lagging or stretched chromatid has been left outside of the nucleus (Fig. 4F). These chromatids appear unable to decondense and seem to be associated with part of a tripolar spindle (Fig. 4L) that persists after centrosomes have duplicated (Fig. 4R) and after most other spindles have disappeared (Fig. 4L). We also observed metaphase plates that appear to have a higher degree of chromosome condensation (Fig. 4C). Furthermore, we frequently find abnormal anaphase figures showing lagging chromatids (Fig. 4D), like those observed in larval neuroblasts.

We also observed abnormal spindle microtubule organization during this early developmental stage. As the nuclei enter prophase, the asters begin to organize (Fig. 4H) and during metaphase most of the spindles are bipolar, although some of them are broad or wavy (Fig. 4I). During anaphase, some spindles seem to share centrosomes (Fig. 4K,Q) and others appear to be very broad in the mid-zone (Fig. 4J). During telophase large asters can be seen to be nucleated from duplicated centrosomes (Fig. 4L,R). The centrosome cycle does appear to be normal. Descrete centrosomes can be visualized during prophase after they have migrated to opposite poles and each cytoplasmic island has two asso-

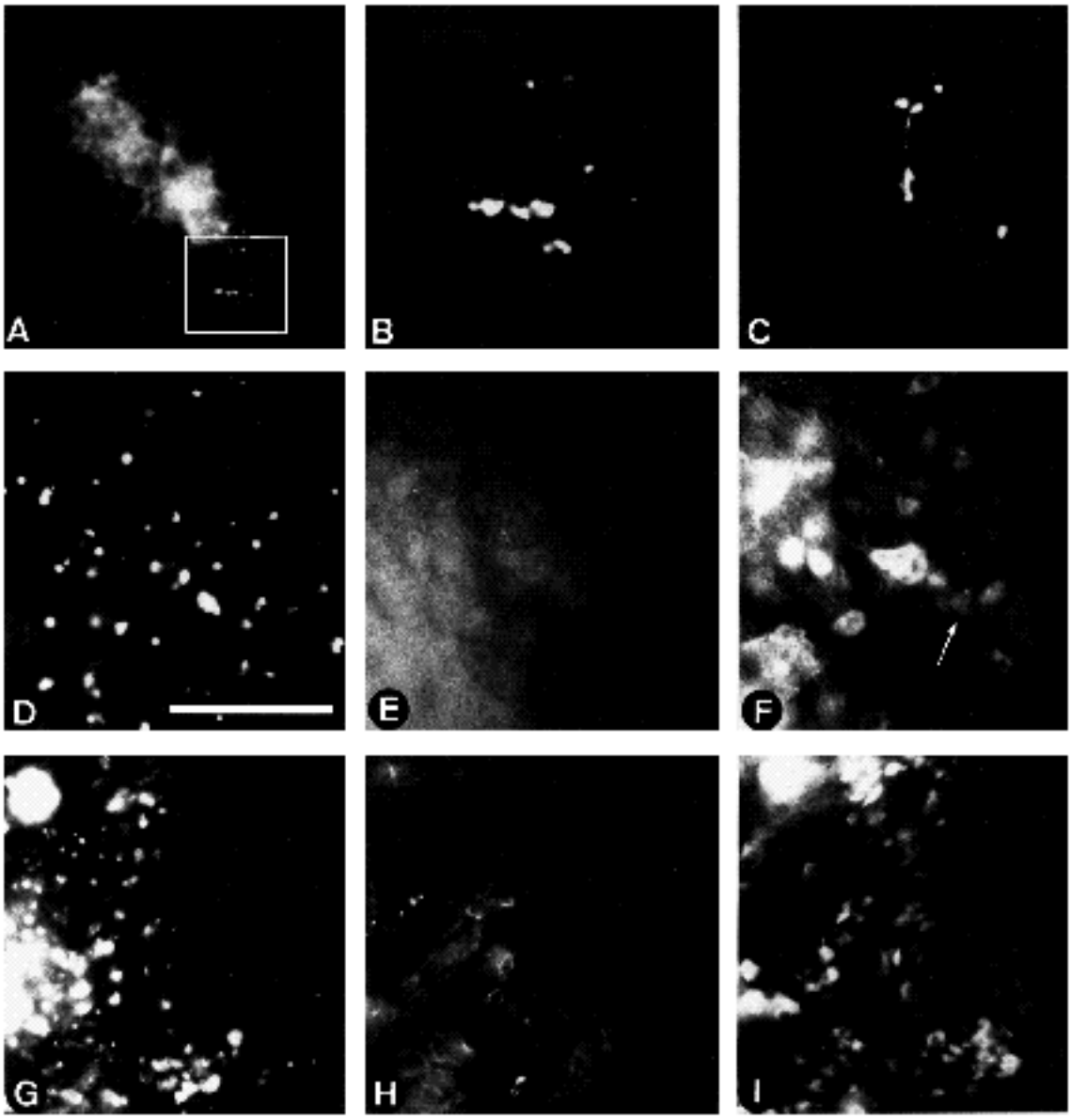

Fig. 5. Abnormal nuclear multiplication stages in embryos produced by *aar*^{2} homozygous females (A-F) and heterozygous *aar*^{2}/Df(3R)by62 females (G-I). Embryos in A, B and C were stained with Hoechst 33258 only. Embryos (D-I) were simultaneously stained with Hoechst 33258 (D,G), anti-tubulin antibody (E,H) and anti-centrosome antibody (F,I). (A) Low magnification view of a very early embryo. (B) Enlargment of the inset in A, showing irregular and highly condensed masses of chromatin. (C) Stretched chromatin associated with irregular and excessive chromatin condensation in a very early embryo. Later multiplication stages (D-I) showing chromatin masses of irregular size dispersed throughout the embryo (D,G) with abnormally arranged cytoplasmic islands, with respect to either tubulin (E,H) or centrosome (F,I) organization. However, some centrosomes can be seen (arrow in F). Bar, 40 μm.

ciated centrosomes (Fig. 4N). Notice that in the absence of nuclei (Fig. 4B-F and N-R), centrosomes in the cytoplasmic islands show a very regular distribution and synchrony.

The embryos obtained from the other two allelic combinations show a somewhat different phenotype. The phenotypes observed in eggs laid by either *aar*^{2} homozygotes (Fig. 5A-C and D-F) or *aar*^{2}/Df(3R)by62 heterozygous females (Fig. 5G-I) are equivalent. In these embryos there are more profound mitotic abnormalities in that most nuclei show irregular DNA content and are abnormally ditributed (Fig. 5A-C). In some of these embryos we also observed chromatin bridges joining two separate and abnormally condensed chromatin masses (Fig. 5C). Older syncytial embryos show similar patterns of disorganized chromatin (Fig. 5D,G). We were unable to observe proper spindle microtubule organization (Fig. 5E,H), and only very occasionally could we see what appeared to be single centrosomes (Fig. 5F).

DISCUSSION

Mutations in the locus *aar* cause mitotic abnormalities during early embryogenesis and late larval development in an allelic-specific manner. *aar*^{1} was identified from a collection of P-element-induced late larval lethals. This mutation was mapped by overlapping deletions to the cytogenetic interval 85E7-F16 and *in situ* hybridization revealed the presence of a single P-element in this region. *aar*^{1} homozygotes have small brains and die during late pupal stages. These individuals probably survive to late developmental stages due to the presence of the maternally provided *aar*$^+$ product. *aar*^{1} causes a range of mitotic abnormalities during metaphase and anaphase in the third-instar larval neuroblasts. The abnormal metaphase figures are characterized by excessive chromosome condensation, low level of polyploidy and in some cases the presence of irregular chromatid condensation. These abnormal phenotypes are probably not due to the absence of functional spindles, since anaphases can be easily found in these brains. However, most anaphases appear abnormal. They are characterized by the presence of stretched chromatids, which extend all the way between the poles, and/or lagging chromatids, which are left in the mid-zone between the two poles. Some anaphases also show variable degree of chromosome condensation. Since we find no extensive chromosome fragmentation at metaphase, it seems unlikely that these abnormal anaphase figures are the result of aberrant DNA replication.

Quantitative analysis of the mitotic activity of wild-type and *aar*^{1} homozygote brains indicates that this mutation causes an increase in the mitotic index. The high mitotic index is due to an increase in both metaphase and anaphase figures, but the ratio of metaphase to anaphase is somewhat reduced relative to wild type. The data suggest that the *aar*^{1} mutation causes a delay in the initiation of anaphase, which results in the large number of metaphase figures with condensed chromosomes. Analysis of brains from *aar*^{1}/Df heterozygotes supports this conclusion and also suggests that the *aar*^{1} allele is leaky. In *Drosophila* neuroblasts, the absence of a mitotic spindle causes cells to spend more time at metaphase, which in turn leads to high chromosome condensation (Gonzalez et al., 1991). However, a more significant mitotic delay in the *aar*^{1} mutant cells might be associated with anaphase progression, because of the increase in the frequency of anaphase figures. This delay is most certainly related to the presence of stretched or lagging chromatids, which is observed in more than 80% of anaphases. Another *Drosophila* gene which shows stretched or lagging chromatids is *rough deal* (Karess and Glover, 1989). Even though mutations in this gene show a very similar phenotype to *aar*^{1} during anaphase, it does not show a delay in metaphase-to-anaphase transit, or a high level of chromosome condensation. However, mutations which display delayed progression from metaphase to telophase have also been observed in other systems. A CHO ts mutant cell line shows, at the restrictive temperature, extensive delay in mitotic progression, which has been correlated with abnormally high levels of histone H1 in late mitosis (Tsuji et al., 1992).

Another mutant allele of the *aar* locus was identified as a recombinant from Df(3R)G42^{P}R36^D. Meiotic recombination and deficiency mapping indicate that *aar*^{2} is also located within the 85E7-F16 interval. However, *aar*^{1}/*aar*^{2} trans-heterozygotes are viable and do not cause abnormal mitotic phenotype in the third-instar larval neuroblasts. *aar*^{2} homozygotes or *aar*^{2}/Df heterozygotes are also viable but females are completely sterile. The results of crosses between males and females of the various allelic combinations indicate that *aar*^{1} affects primarily somatic cells during late larval stages. However, in combination with *aar*^{2} it also affects early embryonic development, although to a lesser extent, since viable adults can be obtained from these females. *aar*^{2} seems to affect only a maternal function because no somatic abnormalities can be observed. Furthermore, the fact that wild-type females fertilized by any of the mutant combinations can give rise to viable progeny indicates that the *aar* mutant alleles do not significantly affect male meiosis.

The mitotic abnormalities observed in eggs laid by either homozygous *aar*^{2} or heterozygous *aar*^{2}/Df females are very severe. These embryos show very abnormal nuclear multiplication, including irregular size and distribution of nuclei, as well as abnormal chromatin condensation. We were never able to observe normal microtubule organization even though centrosomes were occasionally found associated with individual cytoplasmic islands.

The majority of the eggs laid by *aar*^{1}/*aar*^{2} females never complete cellularization. We have characterized the mitotic cycles of these embryos during the nuclear multiplication stage. Indirect immunofluorescence was used to assess chromosome structure and behaviour, spindle microtubule organization and centrosomes. The results indicate that while mitotic synchrony in these embryos is maintained, they develop abnormal nuclear multiplication very early in their development. Large regions of the embryo are devoid of nuclei or they appear with irregular distribution around the cortex. The regions in which no nuclei are found are characterized by the presence of microtubule asters nucleated by individual centrosomes. These centrosomes are associated with well-defined cytoplasmic islands. This phenotype has also been observed in other mitotic mutants

(Freeman et al., 1986; Gonzalez et al., 1990; Vessey et al., 1991), embryos treated with aphidicolin (Raff and Glover, 1988) and after UV irradiation or DNA injection of early embryos (Yasuda et al., 1991). Other mitotic abnormalities are equivalent to those described for the larval neuroblasts. We were also able to observe the fate of lagging chromatids after the nuclei enter telophase. It is surprising that lagging chromatids which are left outside telophase nuclei do not decondense in synchrony with other nuclei and seem to remain attached to spindle microtubules.

Some aspects of the mitotic apparatus do appear to behave normally. Analysis of centrosome behaviour suggests that they follow a normal cycle as described by Callaini and Riparbelli (1990). However, tubulin staining reveals that some mitotic spindles are abnormal. During metaphase abnormal spindles display a wavy and elongated appearance. A similar phenotype was described for embryos produced by a particular class of *asp* alleles (Gonzalez et al., 1990). Other spindles are very broad at the mid-zone, and others seem to share centrosomes. Comparable spindle abnormalities were reported after LLC-PK cells had been exposed to low concentrations of okadaic acid, an inhibitor of protein phosphatases. In those cells, anaphases were never observed and after extended periods of treatment a small proportion of multinucleated cells were found (Vandré and Wills, 1992). In contrast, anaphases were found in both aar^1/aar^1 neuroblasts or embryos laid by aar^1/aar^2 females.

Abnormal spindles were also found in association with lagging chromatids during nuclear multiplication. We frequently found that lagging or stretched chromatids appear to be associated not only with the microtubules nucleated by the opposite centrosomes in that cytoplasmic island, but also from a centrosome located in an adjacent cytoplasmic island. Thus, these spindles are tripolar and it would seeem that lagging or stretched chromatids can establish interactions with microtubules with more that two centrosomes at least within the syncytial embryo. This could not occur with lagging or stretched chromatids in the third larval neuroblasts, unless more than two centrosomes are present in these cells. However, another possibility could be that in the aar^1 mutant cells chromatids can establish functional associations with microtubules from opposite poles, rather than with a single centrosome, the mechanism believed to ensure correct chromosome segregation (reviewed by Gorbsky, 1992). In this event, the aar^+ gene product might be either a structural and/or regulatory component of the mechanism that ensures the maintenance of the correct interaction between spindle microtubules and the kinetochore. In its absence, interaction between the mitotic apparatus and the chromatid is not established, leading to excessive chromosome condensation and alterations in chromatid distribution and integrity, which result in a general delay in progression through mitosis.

The initial stages of this work were carried out in Imperial College, London, with the support of a Medical Research Council Grant to D.M.G. and R.E.K. Subsequent work in the UK was supported by grants from the Cancer Research Campaign and the Human Frontiers Science Programme to D.M.G. and H.O. The work in Porto was supported by a Grant from the Junta Nacional de Investigação Científica e Tecnológica (PMCT) to CES and the Instituto Nacional de Investigação Científica. We thank Isabel Salazar for immunofluorescence preparations and Adelaide Santos for general technical assistance.

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(Received 25 August 1992 - Accepted 13 October 1992)